V. Report on the Technical Reports Review Subcommittee (TRR Subcommittee) Meeting

Dr. Hailey summarized the meeting held on February 17-18, 2004. Four studies reviewed at the meeting related to the toxic equivalency factor (TEF) initiative involving polychlorinated biphenyls (PCBs), which are dioxin-like compounds, and polychlorinated dibenzofurans (PeCDFs). The validity of the use of TEFs for cancer risk assessment is uncertain. The objective of these studies is to determine the toxicity and carcinogenicity of individual PCBs, dioxin (TCDD), and mixtures of these compounds by (1) determining the potency of the dioxin-like compounds, (2) testing the validity of the TEF method for predicting carcinogenicity of a simple mixture, and (3) determining if non-dioxin like PCBs antagonize the carcinogenicity of dioxin-like PCBs.

The first four reports in the TEF project evaluated 3,3',4,4',5-pentachlorobiphenyl (PCB 126), TCDD, 3,3',4,4',5-pentachlorodibenzofuran **(**PeCDF), and a mixture of the PCB 126, and PeCDF. Three reports on PCBs will follow in Fall 2004 and one in the future. All the studies reported on at the meeting in February were conducted in female Sprague Dawley rats. Dr. Hailey summarized the findings from these studies. There was *clear evidence of carcinogenicity* of TCDD and PCB 126 based on hepatocellular adenomas and cholangiocarcinomas of the liver, benign lung tumors, and squamous carcinomas of the oral cavity. Tumors were also found in the uterus of female rats exposed to TCDD. There was *some evidence of carcinogenicity* of PeCDF based on liver and oral cavity tumors in female rats. There was *clear evidence* of carcinogenicity in rats fed the PCB126/ PeCDF mixture. The TRR Subcommittee approved the findings from each report and further analysis is underway to assess the TEF concept.

The data from the studies on malachite green and its reduced metabolite, leucomalachite green, a dye used in fish farming as an antifungal agent, followed the TEF studies. Malachite green and leucomalachite green were administered in feed to F344 rats and B6C3F1 mice. Malachite green was not tested in males. There was *equivocal evidence of carcinogenicity* based on neoplasms of the thyroid gland, liver and mammary gland of female rats. There was *no evidence* in female mice that malachite green is carcinogenic. Data were *equivocal* for leucomalachite green in rats based on thyroid and testis neoplasm in males and there was *some evidence of carcinogenicity* in female mice based on liver neoplasms.

The report on the carcinogenicity of anthraquinone was re-evaluated in light of comments received from industry suggesting that the presence of a contaminant in the anthraquinone tested may have affected the results of the study. The TRR Subcommittee accepted the report and conclusions as valid for the substance tested; however, there was a tie vote broken by the chair that the title be changed to "anthracene-derived anthraquinone" instead of simply anthraquinone. The TRR Subcommittee asked that the Board revisit the issue of contaminants in NTP study materials.

The NTP conducted studies in two small fish species, the Guppy and Medaka, to evaluate the use of a fish as a model for carcinogenicity. The NTP tested three compounds, two

mutagens and one non-mutagen, which had been tested previously in mice and rats. Because tumors in fish species are found primarily in the liver, the NTP chose carcinogenic chemicals that affect multiple tissues in rodents to determine whether other sites were responsive in the fish. The loss of fish due to early death and their subsequent cannibalization by live fish were problems that interfered with interpretation of the studies in some cases. The results of the fish studies were:

- 2,2, bisbromoethyl-1, 3-propanediol resulted in tumors in multiple tissues when fed to male and female rats and mice. The results were positive in the male Guppy and male Medaka based on liver tumors, but negative in female Medaka. Results were inadequate in female Guppy due to reduced survival.

- The mulit-site rodent carcinogen 1,2,3-trichloropropane was positive in both sexes of both species of fish with liver tumors in all species as well as gallbladder tumors in male and female Medaka.

- Nitromethane, when inhaled, was carcinogenic in male and female mice and female rats, but not male rats. The data were inadequate in the male Guppy (due to survival), and negative in the Medaka and female Guppy.

Dr. Hailey said the NTP was disappointed with the outcome of the fish studies, but added that these assays, limited to three chemicals, may not necessarily be a good assessment of the fish models. He indicated that early death and subsequent cannibalism were problems; the studies were not as inexpensive or as short as originally estimated, and that cost savings in pathology was less than anticipated. In most cases tumors were only found after 12 to 16 months of exposure. Both the Guppy and the Medaka seem less sensitive than rodents although the Medaka appear to be more sensitive than the Guppy The liver was the only target organ in which tumors were found in the fish, except for one case of gall bladder tumors.

Reviews of the following reports will take place at the upcoming December peer review: three TEF studies: 2,2',4,4',5,5'-hexachlorobiphneyl (PCB 153), a mixture of 3,3',4,4',5-pentachlorobiphenyl (PCB 126) and PCB 153, a mixture of 2,3,4,4',5, pentachlorobiphenyl (PCB 118) and PCB 126, sodium chlorate, benzophenone, bromodichloromethane, a transplacental azidothymidine (AZT) study, and a genistein dose range finding study.

1. Public Comment on Anthraquinone

Mrs. Linda Beckett, a private citizen, discussed the use of two preparations of goose repellents that contained 50% 9,10-anthraquinone in her neighborhood in Warrington, VA. Arkion and Airopel, a subsidiary of Arkion, market these preparations known as Flight Control Plus and Avipel. School playgrounds and lakesides in their area are overrun with Canadian geese. Homeowners in the area do not want to build fences or allow grass to grow because these activities reduce property values and esthetics. The chemical repellent has been successful in controlling the numbers of geese that

congregate in their development. However, the downside to spraying these repellents is exposure of the public to these preparations. She is concerned about the safety of these preparations and does not understand why Flight Control Plus is restricted for sale and can only be applied by licensed applicators whereas Avipel sales are unrestricted and unregulated, although the active ingredient is the same in both preparations. She read the original NTP technical report on anthraquinone (TR-494) showing clear evidence of carcinogenicity in mice and rats. However, the revised technical report appears to only address anthraquinone manufactured from anthracene. She obtained a copy of the New York State Department of Environmental Conservation registration disallowing the use of anthraquinone in public areas where people could be exposed by absorption of anthraquinone through the skin. In February 2004, she contacted a representative of Arkion who said that there is clear evidence of carcinogenicity of anthraquinone in mice and rats and her concern about exposure was well grounded. Since the NTP report now limits the conclusion of its study to "anthracene-derived anthraquinone," the company has changed its mind about the toxicity of its product. Now, the company says that only "anthracene-derived anthraquinone" may be carcinogenic, but their product, Flight Control Plus, that contains a plant-derived anthraquinone, is completely safe. She is confused and asked whether Arkion is correct, whether New York State is overacting, and whether this chemical is safe. She questioned whether the company changed the name of their products and distribution to skirt EPA regulations. She said Arkion asked EPA to relax their restrictions, but EPA denied the request until the company produces evidence showing that its chemical is not carcinogenic. She wants to know if these repellents are safe for frequent use and whether children are at risk.

Board Discussion

Dr. Roberts, a member the TRR Subcommittee, summarized the discussion that ensued at the February meeting. He said the anthraquinone used in the 2-year bioassay contained a contaminant, 9-nitroanthracene that is mutagenic. According to Arkion when 9-nitroanthracene is extracted from anthraquinone, the residual anthraquinone is no longer mutagenic. Apparently, 9-nitroanthracene is produced during the manufacture of anthraquinone when anthracene is the starting material. The company maintains that mutagenicity is not observed with the anthraquinone they sell, as it is manufactured by a different process. The TRR Subcommittee had extensive discussion about how to handle the report and whether different sources of anthraquinone should be discussed in the report. A potential solution was to designate the starting material in the manufacturing process of anthraquinone in the name of the report. The recommendation to rename the report to "anthracene-derived anthraquinone" passed by the chair, breaking a tie. He said the TRR Subcommittee did not realize the ramifications of its decision to rename the technical report, which apparently has resulted in the study being marginalized as discussed by Mrs. Beckett. At the end of the meeting, members of the TRR Subcommittee asked that the issue of contaminants be revisited, especially in relation to the possibility that a contaminant, and not the chemical being tested, is responsible for carcinogenicity. Dr. Roberts asked the members of the TRR Subcommittee at the Board meeting to comment on its decision regarding the report's title.

Dr. Klaunig said he attended the meeting and voted to change the title of the report but now he has second thoughts. The results of the bioassay are based on anthracene-derived anthraquinone, which contains 9-nitroanthracene, a mutagenic contaminant. Evidence presented at the public meeting by Arkion suggested that this contaminant might be responsible for the carcinogenicity. However, no carcinogenicity studies have been performed with 9-nitroanthracene. Dr. Klaunig said he is hesitant now about maintaining the title change for the report. He believes the bioassay was conducted correctly.

Dr. Boekeheide said he was present at the meeting and voted against changing the title. He pointed out that there was no clear resolution whether carcinogenicity was due to the contaminant that arose from the manufacturing process, or alternatively was from a metabolite of anthraquinone that had been isolated from the urine of rats fed anthraquinone. He said there was confusion as to the concentration of 9-nitroanthracene in the anthraquinone tested in the bioassay. 9-Nitroanthracene is a bacterial mutagen, but not a known carcinogen in mammals. In addition, he noted that the anthraquinone metabolite found in the urine was found to be several fold more mutagenic than the 9-nitroantharcene found at a concentration of 0.1% in the material tested. For these reasons, he did not vote for a change in name of the report. Dr. Birt said she voted against changing the title of the report for reasons discussed at the meeting and because she thought the report on the carcinogenicity of anthraquinone would be ignored.

Dr. Storer stated that he had originally suggested a change in title of the report because the Subcommittee was at an impasse. The scientific presentation had not proven the source of the carcinogenic activity. He had naively thought that responsible agencies would correctly interpret the information, but he realizes the translation to users has been misused and information has been taken out of context. Regulators and the public have erred on the intent of the name change. He still thinks it is appropriate to characterize and identify correctly what was tested. Dr. Storer said that the information presented at the TRR Subcommittee meeting was complex, but at the time the information was compelling that highly mutagenic contaminants could be reproducibly isolated from anthraquinone manufactured from anthracene. Dr. Andrews voted for the name change and he agreed with the points Dr. Storer made. Dr. Piegorsch voted in favor of a name change because Dr. Storer's suggestion was the best recommendation presented at the meeting; however, he now realizes that the consequences seem to be more far reaching than he expected.

Dr. Mary Anna Thrall who chaired the meeting and broke the tie said she voted for a name change because she thought this would be a special name change only.

Dr. Popp said one has to consider the generic issue of impurities and the generic issue of metabolites make the problem even more complex. He asked for clarification on the degree of contamination and the amount of the major metabolite produced. Dr. Bucher responded that the major metabolite, 2-hydroxyanthraquinone, comprises 40-50% of the metabolites and it is mutagenic. The anthraquinone used in the bioassay was 99.9% pure and it had less than 0.1% of 9-nitroanthracene. The NTP used this particular

anthraquinone because technical grade anthraquinone is only 80-85% pure and the NTP wanted to use a purer material.

Dr. Morandi asked whether the amount of the impurity and its potency could explain the number and type of tumors. She suggested that if the tumors could not be attributed to the impurity then it could not be the cause of the tumors. Dr. Bucher responded that the impurity has been tested only in mutagenicity studies and not in carcinogenicity studies; therefore, this determination cannot be made.

Dr. Popp said the presence of impurities is not unique at all and many chemicals studied in the bioassay have higher levels of impurities. Dr. Bucher said the NTP is satisfied if they can find a chemical that is 99.9% pure and the program attempts to identify any impurity that is present at a level of 0.1% or higher. Dr Popp reiterated that this issue is generic and wondered why the naming of the anthraquinone report should be handled any differently from any other chemical with impurities that has been tested.

Dr. Cohen said nitro aromatic compounds are frequently highly mutagenic in the Ames test and other genotoxicity assays, but there is no correlation of bacterial mutagenicity with mammalian carcinogenicity by these compounds. He would be surprised if the 9-nitroantharcene explained the carcinogenicity of the anthraquinone, since there was clear evidence of carcinogenicity and not a marginal response. Dr. Popp agreed with Dr. Cohen especially since the level of the contaminant is so low.

Dr. Walker asked whether the Board is being asked to approve the recommendation and Dr. Portier responded that the NTP is seeking the Board's advice. It appears to him that the Board is suggesting that this issue of contaminants should be discussed in greater depth with the TRR Subcommittee.

Dr. Klaunig agreed with Dr. Portier and said he would feel more comfortable if the issue of contaminants was discussed further by the Subcommittee. This will be an important consideration when the NTP begins to investigate and define mixtures. Dr. Roberts agreed with Dr. Klaunig and Dr. Popp. The TRR Subcommittee should discuss at what level a contaminant might affect the results of a study and whether that could substantially affect the interpretation of a report. Dr. Popp suggested that the example of anthraquinone could be taken as a basis to look at the broader issue of the interpretation of studies with impurities. He previously served on the Subcommittee and although contaminants have been mentioned in relation to other study chemicals, this issue was not discussed further.

Dr. Storer said it is also an issue of communication and interpretation because regardless of whether the report is entitled anthraquinone or "anthracene derived anthraquinone" the supporters of the safety of non-anthracene derived anthraquinone are stating that the carcinogenicity of anthraquinone is associated with these mutagenic contaminants. This is important for interpretation by state agencies, and the NTP report should be more explicit in discussing the implications of these findings with anthracene-derived anthraquinone. The burden of proof should be on industry to show that other sources of

anthraquionone are safe rather than the NTP having to prove that other sources of anthraquinone are also carcinogenic.

Dr. Carpenter stated that from a regulatory standpoint if he is presented with information on a bioassay in which a compound tested positive, he has to assume that carcinogenicity or toxicity will occur even if the commercial product is only 85% pure.